FEBS Letters 587 (2013) 3354–3359

FEBS Letters

journal homepage: www.FEBSLetters.org

Nuclear-encoded chloroplast RNA polymerase sigma factor SIG2 activates chloroplast-encoded phycobilisome genes in a red alga, *Cyanidioschyzon merolae*

Gaku Fujii [a,b], Sousuke Imamura [a,c], Mitsumasa Hanaoka [b], Kan Tanaka [a,c,*]

[a] *Chemical Resources Laboratory, Tokyo Institute of Technology, 4259-R1-29 Nagatsuta, Midori-ku, Yokohama 226-8503, Japan*
[b] *Graduate School of Horticulture, Chiba University, 648 Matsudo, Matsudo, Chiba 271-8510, Japan*
[c] *Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Saitama 332-0012, Japan*

ARTICLE INFO

Article history:
Received 11 July 2013
Revised 23 August 2013
Accepted 23 August 2013
Available online 10 September 2013

Edited by Ulf-Ingo Flugge

Keywords:
Chloroplast
Light-harvesting
Phycobilisome
Sigma factor
Transcriptional regulation
Cyanidioschyzon merolae

ABSTRACT

The phycobilisome (PBS) is a photosynthetic light-harvesting complex in red algae, whose structural genes are separately encoded by both the nuclear and chloroplast genomes. While the expression of PBS genes in both genomes is responsive to environmental changes to modulate light-harvesting efficiency, little is known about how gene expression of the two genomes is coordinated. In this study, we focused on the four nuclear-encoded chloroplast sigma factors to understand aspects of this coordination, and found that SIG2 directs the expression of chloroplast PBS genes in the red alga *Cyanidioschyzon merolae*.

1. Introduction

The phycobilisome (PBS) is a photosynthetic antennae pigment complex found in cyanobacteria and red algae for the efficient harvesting of light energy. The PBS is a macromolecular complex on the thylakoid membrane composed of core allophycocyanin (APC) proteins and stacked rod proteins such as phycocyanin (PC) and phycoerythrin (PE), and mainly transfers captured light energy to the reaction center of photosystem II (PSII) [1]. The abundance and composition of PBSs is modulated by a number of environmental parameters such as light intensity, light wavelength and nutrient availability [2–5]. As the underlying mechanisms, the expression of PBS genes has been suggested to respond to relevant environmental changes in cyanobacteria [4,6], while little information is available for red algae.

In red algae, PBS genes are complementarily encoded by both the nuclear and chloroplast genomes (see Table S1 for gene distribution in *Cyanidioschyzon merolae*). This architecture is considered to be a result of the endosymbiotic origin of the chloroplast and the subsequent transfer of chloroplast genes to the nuclear genome. However, encoding by two separate genomes requires fine-tuning of the two independent gene expression systems, the mechanism of which still remains largely unknown. Nuclear-encoded PBS genes are transcribed by the nuclear RNA polymerase II as in other eukaryotes, while chloroplast genes are transcribed by a bacterial-type chloroplast RNA polymerase descended from the cyanobacterial endosymbiont. This bacterial-type chloroplast RNA polymerase is likely responsible for all chloroplast transcription in unicellular algae, since there are no evidence of other types of chloroplast RNA polymerase in *C. merolae* as was found in seed plants [7]. In general, the subunits of the chloroplast RNA polymerase core enzyme are encoded by the chloroplast genome, whereas the specificity and transcription initiation factor, sigma, is encoded by the nuclear genome [8]. Thus, the nucleus controls chloroplast transcription through modulation of the expression and/or activity of sigma factor(s), which could be the coordination mechanism between the two genomes for PBS gene expression.

Abbreviations: bp, base pair(s); EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobase(s); kDa, kilodalton(s); OD, optical density; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; SD, standard deviation; SDS, sodium dodecyl sulfate

* Corresponding author at: Chemical Resources Laboratory, Tokyo Institute of Technology, 4259-R1-29 Nagatsuta, Midori-ku, Yokohama 226-8503, Japan. Fax: +81 45 924 5274.
E-mail address: kntanaka@res.titech.ac.jp (K. Tanaka).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.08.031

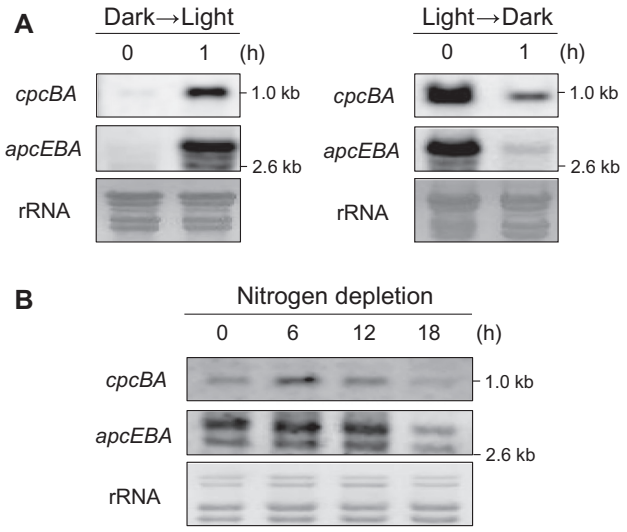

Fig. 1. Expression of chloroplast PBS genes under light/dark shifts and nitrogen depletion. (A) Changes of *apcEAB* and *cpcBA* transcript levels under light/dark shifts. *C. merolae* cells were harvested at the indicated times after dark-to-light or light-to-dark shift, and total RNAs were prepared. The RNAs (3 μg) were then subjected to Northern blot analysis with specific probes. The positions of molecular weight markers are shown on the right. The electrophoretic patterns of rRNAs stained with methylene blue are shown below as loading controls. (B) Changes of *apcEAB* and *cpcBA* transcript levels during nitrogen depletion. RNAs (1.5 μg) were subjected to Northern blot analysis as in (A).

C. merolae is a unicellular red alga that lives in acidic high-temperature environments (pH 1–3, 40–50 °C) and was isolated from an Italian hot spring [9]. Because of the extremely primitive features of the cell, *C. merolae* has been the subject of various basic analyses including elucidation of the complete genome sequences of the nucleus, chloroplast and mitochondrion [10–13], and the development of tools and systems for molecular genetics analyses [14–16]. Based on the nuclear genome sequence, four chloroplast sigma factor genes have been identified and designated *SIG1–4* (http://merolae.biol.s.u-tokyo.ac.jp/) [17]. In this study, we focused on and analyzed these nuclear-encoded sigma factors to understand the nuclear control of chloroplast PBS gene expression in red algae, and found that SIG2 directs the expression of chloroplast PBS genes.

2. Materials and methods

2.1. Strain and growth conditions

C. merolae 10D was cultivated in liquid MA2 medium [14] under continuous white light (50 μmol m^{-2} s^{-1}) at 40 °C, bubbled by air supplemented with 2% CO_2. For the M4 strain, 0.5 mg/mL uracil was added to the medium. Nitrogen depletion condition was as described in [15].

2.2. Construction of SIG overexpression and underexpression strains

Details of the construction of the *SIG2* overexpression and underexpression plasmids are described in the Supplementary methods. Transformation of the M4 strain was performed as described previously [14–16].

2.3. Immunoblot analysis

Immunoblot analysis was performed as described previously [18]. Antibodies against SIG1–4 were as described [19].

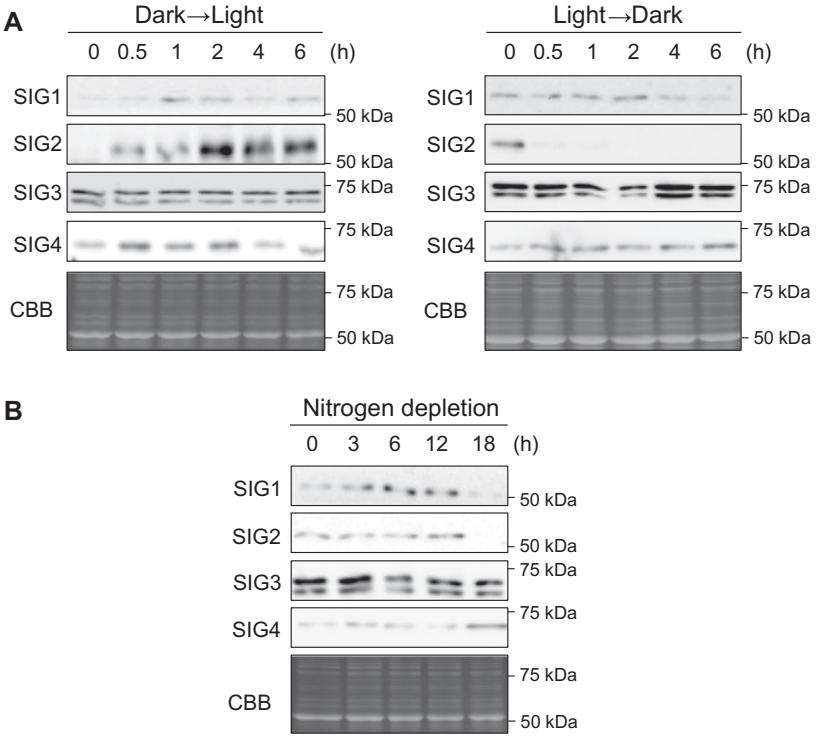

Fig. 2. Accumulation of sigma factor proteins under light/dark shifts and nitrogen depletion. (A) Protein levels of SIG1–4 during dark-to-light or light-to-dark shifts. *C. merolae* cells were harvested at the indicated times after dark-to-light or light-to-dark shifts. Aliquots containing 10 μg soluble protein from *C. merolae* cells were separated by 10% SDS–polyacrylamide gel electrophoresis (PAGE) and analyzed by immunoblot analysis with antibodies specific for each sigma (SIG1–4). The positions of molecular weight markers are shown on the right. Loaded proteins stained Coomassie Brilliant Blue (CBB) are shown below as a loading control. (B) Protein levels of SIG1–4 under nitrogen depletion. All other figure elements are as described in A.

2.4. RNA extraction and Northern blot analysis

Total RNA extraction and Northern blot analysis were performed as described in [18]. Probes for Northern blot analysis were prepared as in [18] with the specific primers described in Table S3.

2.5. Chromatin immunoprecipitation (ChIP) analysis

Logarithmically grown *C. merolae* cells (optical density $(OD)_{750}$ = 0.4–0.7, 25 mL) were fixed with 1% formaldehyde at room temperature for 10 min with gently mixing. Subsequently, glycine was added to the cells at final concentration of 0.125 M and the cells were gently mixed for 5 min. The cells were then pelleted, washed by ice-cold phosphate-buffered-saline (8.1 mM Na_2PO_4, 1.47 mM KH_2PO_4, 137 mM NaCl, 2.68 mM KCl), and stored −80 °C until use. The pellets were resuspended in 1 mL sodium dodecyl sulfate (SDS) lysis buffer (50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–KOH, 140 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% SDS, pH 7.5, Complete Mini, EDTA-free, protease inhibitor), and cell disruption and shearing of genomic DNA were achieved by sonication. The genomic DNA was fragmentized from 500 to 850 base pair (bp). Others methods were as described in [18]. The primers for quantitative real time PCR are listed in Table S4.

2.6. Quantitative analysis of phycocyanin

Phycocyanin contents were calculated based on OD_{620}, OD_{678} and OD_{750} basically as described in [20].

3. Results

3.1. Expression analysis of PBS genes in C. merolae

Previous reports indicated that the expression of PBS genes responds to external light and nitrogen conditions in cyanobacteria [4,6,21]. To ascertain that this was also the case in *C. merolae*, we analyzed the transcript levels of chloroplast-encoded PBS gene clusters, *cpcBA* and *apcEAB*, each of which presumably comprises an operon, during shifts in light or nitrogen conditions. Both transcripts were found to be induced by the onset of light and decreased by a dark shift (Fig. 1A). Nitrogen depletion resulted in a transient increase and a subsequent decrease of the transcripts (Fig. 1B), suggesting that the PBS gene expression is regulated in *C. merolae* chloroplast similarly as in cyanobacteria [4,6,21].

3.2. Correlation of chloroplast PBS gene expression and accumulation of SIG proteins

If transcription of chloroplast PBS genes is under the control of a specific nuclear-encoded sigma factor(s), accumulation of the responsible SIG protein(s) should correlate with the accumulation of PBS gene transcripts. Thus, the levels of the four SIG proteins under the same conditions as in Fig. 1 were examined by immunoblot analysis. When *C. merolae* cells were exposed to light after dark adaptation, the SIG2 protein level began to increase at 0.5 h, and subsequently reached a peak at 2 h (Fig. 2A). SIG1 was also increased 1 h after the light shift, and this increased level persisted for at least 6 h. In the case of a light-to-dark shift, SIG2 was significantly decreased at 0.5 h, and was undetectable after 1 h. SIG1 was also decreased by the dark shift, but this decrease was very slow compared with SIG2. Nitrogen depletion also resulted in a decrease of the SIG1 and SIG2 proteins after 18 h. The levels of the SIG3 and SIG4 proteins were not significantly affected by light or nitrogen conditions. These results suggested a correlation between the accumulation of SIG2 and chloroplast PBS gene transcripts, and raised the possibility that SIG2 activates chloroplast PBS gene transcription.

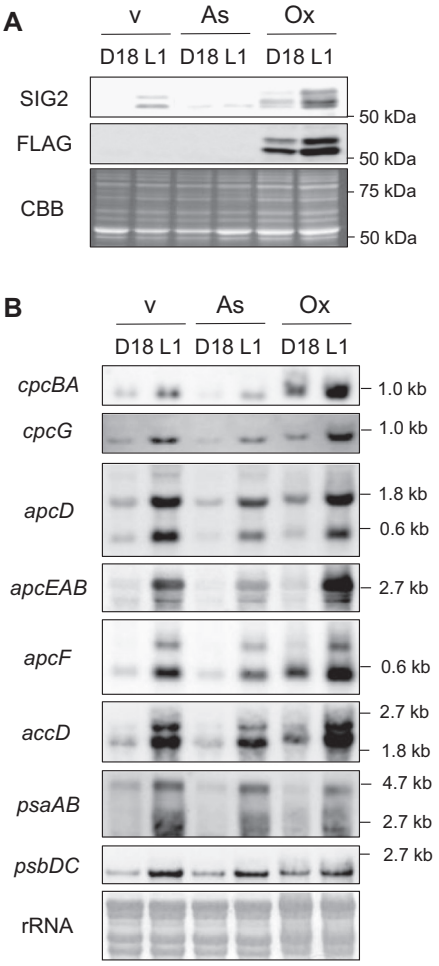

Fig. 3. Effects of SIG2 overexpression and underexpression on chloroplast PBS gene expression. (A) Immunoblot detection of SIG2 in SIG2-v, SIG2-As and SIG2-Ox strains. Accumulation of the SIG2 protein was examined using SIG2-specific antiserum (SIG2) or a monoclonal antibody against the FLAG epitope (FLAG). Strains were dark adapted for 18 h, and harvested before (D18) or 1 h after light illumination (L1). All other figure elements are as described in Fig. 1. (B) Transcript levels of chloroplast PBS and other genes under a dark-to-light shift. SIG2-v, SIG2-As and SIG2-Ox strains were cultivated and harvested as in A. RNAs (3 μg) were prepared and subjected to Northern blot analysis as in Fig. 1A.

3.3. Overexpression and underexpression of SIG2 resulted in correlated changes of chloroplast PBS gene expression

If SIG2 positively regulates chloroplast PBS genes, artificial changes of SIG2 levels should result in correlated changes of chloroplast PBS gene expression. To examine this possibility, the SIG2-coding region was placed downstream of the strong *APCC* promoter in either forward or reverse orientation (pOxSIG2 or pAsSIG2, Fig. S1), and introduced in *C. merolae* cells to make SIG2-sense or -antisense RNA overexpressing strains, SIG2-Ox and SIG2-As, respectively (Fig. S2). To make these strains, plasmid-introduced cells were selected for uracil autotrophy, but the status of the introduced plasmid, i.e., whether it was integrated into the chromosome or transiently replicating autonomously [22–23], was not examined in this experiment. The SIG2-v strain, into which the vector plasmid pSUGA (Fig. S1) was introduced, was used as the control. A dark-to-light shift was shown to induce SIG2 in this control strain (Fig. 3A) as in the wild type (Fig. 2A). On the other hand, SIG2 induction was not observed in SIG2-As but was

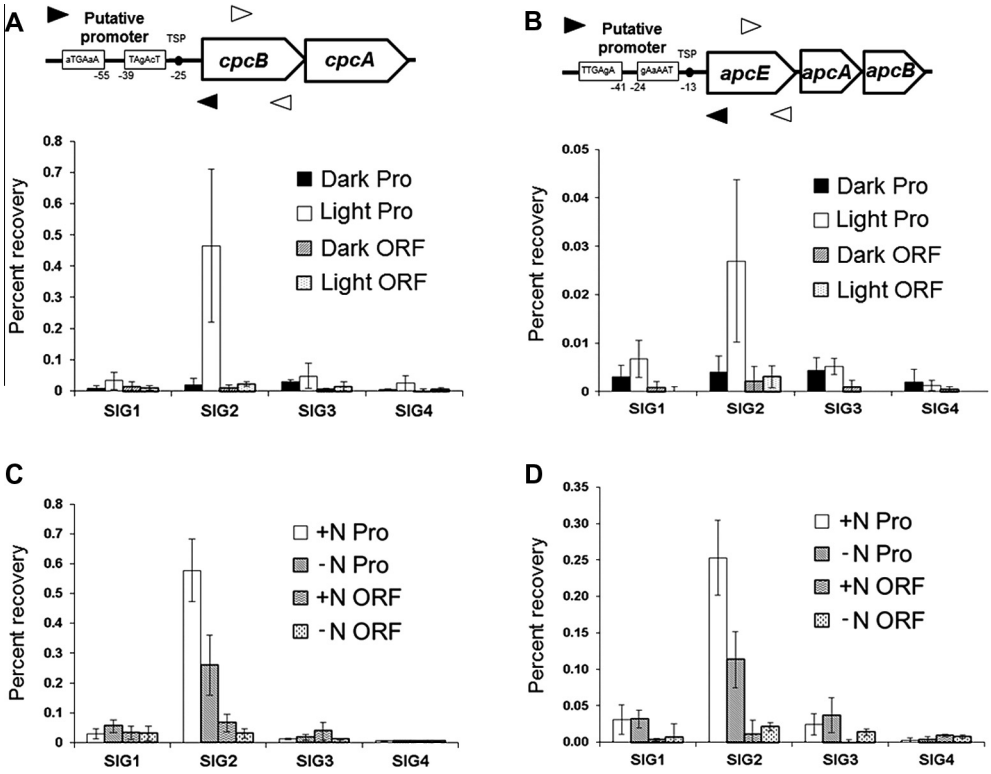

Fig. 4. Specific interaction of sigma factors with chloroplast promoter regions in vivo. *C. merolae* cells were fixed by formaldehyde before or after 1 h light illumination after 18 h dark adaptation (A and B) or fixed before or after 18 h nitrogen depletion (C and D). Sigma factor–DNA complexes were precipitated with SIG1–4 antibodies, and the levels of immunoprecipitated DNA for chloroplast *cpcBA* (A and C) and *apcEAB* (B and D) gene promoters (Pro) or downstream ORF regions were calculated. Sets of black and white arrowheads indicate the positions of primers used to amplify the putative promoter and downstream ORF regions. TSP indicates transcription start site predicted by EST and MPSS analyses. The putative promoters were indicated by boxes with their sequences and positions: capital characters, the consensus nucleotides of the major class of bacterial promoters; +1 as the initiation codon of each gene. All values are averages of three independent experiments and represent the percent recovery relative to the total input DNA. Error bars correspond to ± standard deviation (S.D.).

enhanced in SIG2-Ox by immunoblot analysis with anti-SIG2 antibody (Fig. 3A). The strong induction in SIG2-Ox was also confirmed by immunoblot analysis with the antibody against the FLAG-tag that was fused to the SIG2 carboxy-terminus (Fig. 3A). Two signals were detected by the anti-SIG2 antibody in most cases; the upper band was likely the unprocessed precursor protein with the chloroplast translocalization transit-peptide. In the case of SIG2-Ox, a third faint signal was detected by anti-SIG2 antibody at the lower position. This likely corresponded to the endogeneous mature SIG2 protein without the FLAG-tag. We subsequently examined the transcript levels of the chloroplast PBS genes (Table S1) during the dark-to-light shift. As shown in Fig. 3B, *cpcBA*, *cpcG*, *apcEAB* and *apcF* transcripts were decreased in SIG2-As and increased in SIG2-Ox as compared with SIG2-v. For *apcD*, the transcript level changed only slightly in SIG2-Ox but was decreased in SIG2-As. *accD* is likely comprising an operon with *apcD*, and the transcript levels increased or decreased correlating with other PBS genes. Almost no difference was observed among the three strains for *psaAB*, which encodes P700 apoproteins of PSI, and *psbDC*, which encodes the reaction center D2 and CP43 proteins of PSII. These observations well supported the hypothesis that SIG2 specifically activates chloroplast PBS gene transcription.

3.4. Specific interaction of SIG2 with the promoter regions of the PBS genes

To clarify whether SIG2 directly recognizes chloroplast PBS gene promoters, chromatin immunoprecipitation (ChIP) analysis was performed using antiserum against each SIG protein. Transcription start points (TSPs) of *cpcB* and *apcE* genes were determined by 5′-massively parallel signature sequence (MPSS) analysis (Imamura et al., in preparation) [24]. The TSPs were mapped at 13-bp for *cpcB* and 25-bp for *apcE* upstream from each initiation codon, respectively. The MPSS results were consistent with results obtained by the full-length EST analysis [12]. Sequences that resemble the major class of bacterial promoters [25] were found around 10 and 35 bp upstream of each TSP (Fig. 4A and B). Cells were cultivated under continuous illumination, and then dark adapted for 18 h. The cells were sampled before and after a 1 h illumination, and the presence of each SIG protein on the putative *cpcBA* and *apcEAB* promoter regions was analyzed. Whereas no significant interaction was found under the dark condition, specific SIG2 interaction with these promoter regions was detected after the illumination (Fig. 4A and B). In contrast, these specific interactions of SIG2 to the *cpcBA* and *apcEAB* promoter regions were decreased 18 h after the nitrogen depletion (Fig. 4C and D), in which the PBS gene transcripts and SIG2 protein levels were decreased (Fig. 2B). No significant interaction of SIG2 was found with DNA regions inside the open reading frames (ORFs) of the analyzed genes under any environmental conditions shown in Fig. 4. These results again indicated that SIG2 specifically and directly activates chloroplast PBS genes.

3.5. The SIG2 overexpression affects phycocyanin contents under nitrogen depletion

To further examine the SIG2–PBS relationship, we analyzed phycocyanin content in WT cells under dark to light shift and nitrogen depletion condition. As the result, while no significant change of phycocyanin content was observed by light shifts (data not shown), phycocyanin content significantly decreased by nitrogen depletion. We subsequently analyzed phycocyanin contents of SIG2-v, SIG2-As and SIG2-Ox strains. While there is no significant

difference among SIG2-v, SIG2-As, and SIG2-Ox strains under the nitrogen-replete condition (Fig. 5), reduction of the phycocyanin content was alleviated in SIG2-Ox as compared with SIG2-v and SIG2-As under the nitrogen depletion condition. These results support the idea that the chloroplast encoded PBS-related genes are positively regulated by SIG2.

4. Discussion

The abundance of light harvesting PBS complexes is regulated by various environmental cues in cyanobacteria and red algae. As the underlying mechanism, while transcriptional control of PBS genes has been shown to play a major role in cyanobacteria, little information is available for the regulation in red algae. PBS genes are encoded by both nuclear and chloroplast genomes in red algae, and thus the regulation is likely rather complex as compared with those in cyanobacteria. In *C. merolae*, four PBS genes (*CPCC*, *CPCE*, *CPCF* and *APCC*) are encoded by the nuclear genome and thus must be transcribed in the nucleus and imported into the chloroplast. On the other hand, the expression of the chloroplast-encoded PBS genes (*cpcBA*, *cpcG*, *apcEAB* and *apcF*) is performed totally in the chloroplast compartment. This complicated situation indicates that biosynthesis of PBS requires an intricate mechanism for communication and coordination of the two independent genomes.

In this study, we examined the hypothesis that a nuclear-encoded sigma factor(s) has a role in chloroplast PBS gene transcription and regulation. From our results, we concluded that SIG2 is involved in chloroplast PBS gene transcription because (1) the accumulation of SIG2 and chloroplast PBS gene transcripts was well correlated, (2) overexpression and underexpression of SIG2 resulted in overexpression and underexpression of chloroplast PBS genes, respectively, and (3) ChIP analysis revealed specific interaction between SIG2 and chloroplast PBS gene promoters. These observations were specific for SIG2 among the four sigma factors, indicating a specific functional linkage of SIG2 with PBS. Other than the nuclear-encoded SIG genes, the *C. merolae* chloroplast genome encodes four bacterial-type transcription factors, Ycf27–30 [11]. Involvement of these factors in PBS gene transcription is also possible but presently unknown, and should be clarified in future studies.

Does SIG2 regulate only chloroplast PBS genes or also recognize other chloroplast promoters? While obtaining the full picture will require comprehensive analysis, the lipid biosynthetic *accD* and the PSII component *psbX* genes are transcribed with *apcD* as an operon under the control of SIG2 (Fig. 3B). This observation suggests a more global role for SIG2 in chloroplast gene expression. Other question is contributions of other sigma factor(s) under the dark-to-light shift and nitrogen deprivation conditions. Recently, in higher plants, it has been reported that activity of sigma factors are regulated at the posttranslational level [26]. Thus, it is possible that other sigma factor(s), of which protein levels were not changed in this study, contributes to gene expression under the environmental conditions. This point must be important for the future studies.

In conclusion, we have identified the nuclear-encoded sigma factor SIG2 as a positive regulator of chloroplast PBS genes. This is the first identification of a nuclear factor involved in chloroplast PBS accumulation, and represents the first step in the comprehensive understanding of chloroplast transcription networks in red algae.

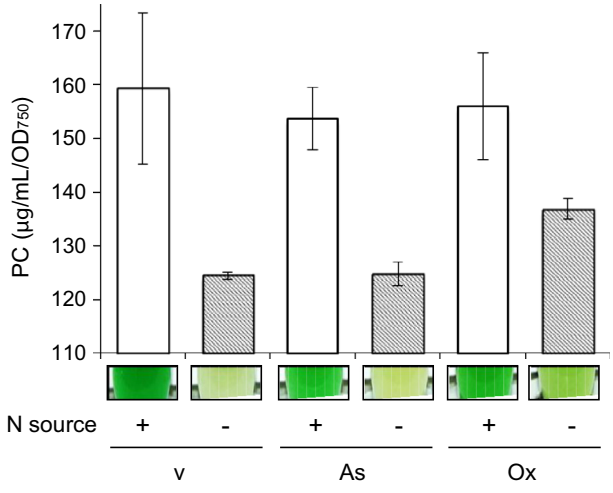

Fig. 5. Changes of phycocyanin content after nitrogen depletion. SIG2-v, SIG2-As and SIG2-Ox strains were harvested at the late logarithmic phase (OD_{750} = 2.0–5.0), and resuspended in MA2 or nitrogen depletion medium as the OD_{750} was adjusted to 0.5. These cells were cultivated in microtiter plates (6 holes) for 48 h and the OD_{620}, OD_{678}, and OD_{750} were measured to estimate the phycocyanin contents (μg/mL/OD_{750}). Values shown are means ±SD derived from three measurements. Significant differences were calculated by Student's *t*-test ($P > 0.05$). Pictures of the cultures after the 48 h cultivation were shown at the bottom of the graph.

Acknowledgments

This study was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grants-in-Aid 21370015, 23120505 and 2424806 to K.T., Grants-in-Aid 24117505, 24770037 and 25120705 to M.H. and Grants-in-Aid 22681010, 24117521 and 25440129 to S.I.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.08.031.

References

[1] Gantt, E. (1981) Phycobilisomes. Annu. Rev. Plant Physiol. 32, 327–347.
[2] Duke, C.S., Cezeaux, A. and Allen, M.M. (1989) Changes in polypeptide composition of *Synechocystis* sp. strain 6308 phycobilisomes induced by nitrogen starvation. J. Bacteriol. 171, 1960–1966.
[3] Vernotte, C., Picaud, M., Kirilovsky, D., et al. (1992) Changes in the photosynthetic apparatus in the cyanobacterium *Synechocystis* sp. PCC 6714 following light-to-dark and dark-to-light transitions. Photosynth. Res. 32, 45–57.
[4] Gutu, A. and Kahoe, D.M. (2012) Emerging perspectives on the mechanisms, regulation, and distribution of light color acclimation in cyanobacteria. Mol. Plant 5, 1–13.
[5] Kawakami, T., Sakaguchi, K., Takeuchi, K., et al. (2009) Ammonium induced expression of the red algal chloroplast gene Ycf18, a putative homolog of the cyanobacterial NblA gene involved in nitrogen-induced phycobilisome degradation. Biosci., Biotechnol., Biochem. 73, 740–743.
[6] Grossman, A.R., Schaefer, M.R., Chiang, G.G., et al. (1993) The phycobilisome, a light-harvesting complex responsive to environmental conditions. Microbiol. Rev. 57, 725–749.
[7] Hajudukiewicz, P.T.J., Allison, L.A. and Maliga, P. (1997) The two type RNA polymerase encoded by the nuclear and the plastid compartments transcribe distinct groups of genes in tobacco plastids. EMBO J. 16, 4041–4048.
[8] Liere, K., Weihe, A. and Börner, T. (2011) The transcription machineries of plant mitochondria and chloroplasts: composition, function, and regulation. J. Plant Physiol. 168, 1345–1360.
[9] Kuroiwa, T. (1998) The primitive red algae *Cyanidium caldarium* and *Cyanidioschyzon merolae* as model system for investigating the dividing apparatus of mitochondria and plastids. Bioessays 20, 344–354.
[10] Ohta, N., Sato, N. and Kuroiwa, T. (1998) Structure and organization of the mitochondrial genome of the unicellular red alga *Cyanidioschyzon merolae* deduced from the complete nucleotide sequence. Nucleic Acids Res. 26, 5190–5198.
[11] Ohta, N., Matsuzaki, M., Misumi, O., et al. (2003) Complete sequence and analysis of the plastid genome of the unicellular red alga *Cyanidioschyzon merolae*. DNA Res. 10, 67–77.

[12] Matsuzaki, M., Misumi, O., Shin-I, T., et al. (2004) Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. Nature 428, 653–657.
[13] Nozaki, H., Takano, H., Misumi, O., et al. (2007) A 100%-complete sequence reveals unusually simple genomic features in the hot-spring red alga *Cyanidioschyzon merolae*. BMC Biol. 5, 28.
[14] Ohnuma, M., Yokoyama, T., Inouye, T., et al. (2008) Polyethylene glycol (PEG)-mediated transient gene expression in a red alga, *Cyanidioschyzon merolae* 10D. Plant Cell Physiol. 49, 117–120.
[15] Imamura, S., Kanesaki, Y., Ohnuma, M., et al. (2009) R2R3-type MYB transcription factor, CmMYB1, is a central nitrogen assimilation regulator in *Cyanidioschyzon merolae*. Proc. Natl. Acad. Sci. USA 106, 12548–12553.
[16] Imamura, S., Terashita, M., Ohnuma, M., et al. (2010) Nitrate assimilatory genes and their transcriptional regulation in a unicellular red alga *Cyanidioschyzon merolae*: Genetic evidence for nitrite reduction by a sulfite reductase-like enzyme. Plant Cell Physiol. 51, 707–717.
[17] Minoda, A., Hanaoka, M., Nagasawa, K., et al. (2005) Microarray profiling of plastid gene expression in a unicellular red alga. *Cyanidioschyzon merolae*. Plant Mol. Biol. 59, 375–385.
[18] Imamura, S., Hanaoka, M. and Tanaka, K. (2008) The plant-specific TFIIB-related protein, pBrp, is a general transcription factor for RNA polymerase I. EMBO J. 27, 2317–2327.
[19] Kanesaki, Y., Imamura, S., Minoda, A., et al. (2012) External light condition and cell cycle phases coordinate accumulation of chloroplast and mitochondrial transcripts in the red alga *Cyanidioschyzon merolae*. DNA Res. 19, 289–303.
[20] Arnon, D.I., McSwain, B.D., Tsujimoto, H.Y., et al. (1974) Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll *a* and removal of phycocyanin. Biochim. Biophys. Acta 357, 231–245.
[21] Sauer, J., Gorl, M. and Forchhammer, K. (1999) Nitrogen starvation in *Synechococcus* PCC 7942: involvement of glutamine synthetase and NtcA in phycobiliprotein degradation and survival. Arch. Microbiol. 172, 247–255.
[22] Ohnuma, M., Misumi, O., Fujiwara, T., et al. (2008) Transient gene suppression in a red alga, *Cyanidioschyzon merolae* 10D. Protoplasma 236, 107–112.
[23] Watanabe, S., Ohnuma, M., Sato, J., et al. (2011) Utility of a GFP reporter system in the red alga *Cyanidioshyzon merolae*. J. Gen. Appl. Microbiol. 57, 69–72.
[24] Brenner, S., Johnson, M., Bridgham, J., et al. (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat. Biotechnol. 18, 630–634.
[25] Harley, C.B. and Reynolds, R.P. (1987) Analysis of *E. coli* promoter sequences. Nucleic Acids Res. 15, 2343–2361.
[26] Türkeri, H., Schweer, J. and Link, G. (2012) Phylogenetic and functional features of the plastid transcription kinase cpCK2 from Arabidopsis signify a role of cysteinyl SH-groups in regulatory phosphorylation of plastid sigma factors. FEBS J. 279, 395–409.